



ANTIBODIES REACTIVE WITH $\beta(1-3)$ -GLUCANS

Technical field

The present invention relates to new antibodies reactive with $\beta(1-3)$ -glucans, as well as to the use of such antibodies.

5 Background of the invention

Fungal infections may occur at many places in the human or animal body, e.g. in the vagina or in the oral cavity.

Invasive fungal infections are increasing because of the growing number of immunocompromised patients (7). Almost all of these infections occur in critically ill
10 patients suffering from an underlying disease.

In *Candida* species which are the most common fungi isolated from patients with invasive fungal infection, the yeast cells are surrounded by a rough, rigid cell wall that represents 20-25% of the dry weight of the cells (9). The cell wall of *Candida albicans* and *Saccharomyces cerevisiae* consists of about 85-90% polysaccharide, 10-15% protein, and a small amount of lipids (19, 20). The polysaccharide components consist of mannan, glucan, and a small amount of chitin. Most of the proteins
15 are covalently linked to the mannan (mannoprotein), which is located in the outermost layer of the cell wall. A fraction of the proteins is also covalently linked to glucan (8). The proportions of these different components vary with the species, but in
20 *Saccharomyces cerevisiae* there are approximately equal proportions of mannan and glucan, and about equal amounts of alkali-soluble glucan and alkali-insoluble glucan (3). The glucan microfibriles are located mostly in the inner part of the cell wall. The high mannose content present in *Candida albicans* cell wall is absent in *Cryptococcus neoformans*, and glucose is the major monosaccharide constituent of the cryptococcal
25 cell wall. The cell wall of uncapsulated *Cryptococcus neoformans* is composed mainly of glucan.

$\beta(1-3)$ -glucans are unique for all medically important fungi and are shed during growth (16). Thus, determination of $\beta(1-3)$ -glucans appear to be a useful marker in the laboratory diagnosis of deep fungal infections.

The analysis of $\beta(1-3)$ -glucans is based on the binding of the polysaccharide to the coagulation factor G. This glucan test, however, has some limitations. It does not react exclusively with $\beta(1-3)$ -glucans, since also $(1-3)(1-4)$ - α -D-glucan (negaran) and $(1-2)(1-3)(1-6)$ - α -D-glucan (yeast α -D-mannan), and $(1-6)$ - β -D-glucan (gyrophoran) may activate the G factor (18). The reactivity of factor G is also dependent on the molecular weight, conformation and degree of branching of the glucans (18). Moreover, there are some contradictions regarding its effectiveness of determining glucans in *Cryptococcus neoformans* infections (17).

Compounds with a high binding specificity for $\beta(1-3)$ -glucans would be useful tools for providing an analysis of $\beta(1-3)$ -glucans in any body fluid, such as blood, urine, or in biopsy specimens of patients with suspected fungal infections, and consequently for providing a laboratory diagnosis of fungal infection.

Summary of the invention

The object of the present invention is to provide antibodies reactive with $\beta(1-3)$ -glucans as present in free form, in cell wall fragments, and in intact fungal cells with a high specificity for use in the laboratory diagnosis of fungal infections.

According to a first aspect of the present invention, a monoclonal antibody reactive with $\beta(1-3)$ -glucans is provided. Said antibody is reactive with $\beta(1-3)$ - and/or $\beta(1-3)(1-6)$ -glucan associated epitopes in free, non-associated form, in cell wall fragments and/or on an intact cell surface. Said antibody is B3B or A10A.

According to a second aspect of the invention, the use of said antibody for the diagnosis of fungal infections is provided. Further, said antibody may be used for the detection of medically important fungi, e.g. mould, in air, water, dust or other components.

According to a third aspect of the invention, a diagnostic kit for the diagnosis of fungal infections comprising said monoclonal antibody is provided.

According to a fourth aspect of the present invention, a method for diagnosing fungal infections comprising said monoclonal antibody is provided. Additionally, a method for detecting medically important fungi, e.g. mould, is provided.

Brief description of the drawings

Figure 1 shows the antibody activities of A10A and B3B to $\beta(1-3)(1-6)$ -glucan, $\beta(1-3)$ -glucan, $\beta(1-3)(1-4)$ -glucan, $\beta(1-6)$ -glucan, *Candida albicans* cell wall fragments (CaCW), and *Cryptococcus neoformans* cell wall fragment (CnCW) as analyzed by ELISA at a dilution of 1/10. The antibody activity is expressed as the absorbance value.

Detailed description of the invention

As stated above, the cell wall of all medically important fungi contains a unique polyglucose compound, a $\beta(1-3)$ -glucan. $\beta(1-3)$ -glucans refer to polysaccharides having the basic unit $\beta(1-3)$. These glucans may be $\beta(1-3)$ -glucans without side chains, or may be branched to various degrees having $\beta(1-6)$ side chains, $\beta(1-3)(1-6)$ -glucans. The side chains may be varied with respect to the number of $\beta(1-6)$ per $\beta(1-3)$, the length of $\beta(1-6)$ branched $\beta(1-3)$ etc.

Murine monoclonal antibodies were produced against linear $\beta(1-3)$ -glucans and $\beta(1-6)$ -branched $\beta(1-3)$ -glucans (also called $\beta(1-3)(1-6)$ -glucan) and their specificity was characterized. The antibodies were analysed for reactivity to other β -glucans, fungal cell wall fragments, and intact fungal cells.

Two monoclonal antibodies, A10A and B3B, reactive with $\beta(1-3)$ -glucan and $\beta(1-3)(1-6)$ -glucan in ELISA, recognized immunoreactive epitopes in *Candida albicans* and non-encapsulated *Cryptococcus neoformans* cell wall fragments (CaCW, CnCW) (fig 1). The A10A epitope was present in both $\beta(1-3)$ -glucan and $\beta(1-6)$ -glucan. The B3B epitope included $\beta(1-3)$ -glucan, but most probably not $\beta(1-6)$ -glucan. Thus, B3B appeared to recognize the $\beta(1-3)$ -linkage, present in $\beta(1-3)$ -glucan and $\beta(1-3)(1-6)$ -glucan, while A10A reacts with glucan consisting of both types of linkages, i.e. reacts with $\beta(1-3)$ -glucans, $\beta(1-6)$ -glucans and $\beta(1-3)(1-6)$ -glucans.

By indirect immunofluorescence only A10A recognized a $\beta(1-3)(1-6)$ associated epitope on the intact cell surface of *Candida albicans*, *Candida parapsilosis*, *Candida krusei*, *Candida glabrata*, and *Cryptococcus neoformans*.

In summary, B3B demonstrated the presence of immunoreactive epitopes, i.e. $\beta(1-3)$ -glucan and $\beta(1-3)(1-6)$ -glucan, in cell wall fragments of *Candida albicans* and *Cryptococcus neoformans* (fig 1), as well as in free form $\beta(1-3)$ - and $\beta(1-3)(1-6)$ -

glucans (table 2), while A10A in addition recognized a $\beta(1-3)(1-6)$ -glucan associated epitope that was readily available on the surface of whole cells of *Cryptococcus neoformans* and all *Candida* species tested.

Thus, the two monoclonal antibodies to $\beta(1-3)$ -glucans, A10A and B3B, could
 5 be used in combination (although not excluding also separately) in an immunoassay for the detection of free, cellwall associated or cell surface associated $\beta(1-3)$ -glucans. Thereby, they are of help in laboratory diagnosis of fungal infections, in particular deep fungal infections, but also superficial infections, such as *Candida* vaginitis or mucocutane candidiasis.

10 Thus, in the research work leading to the present invention murine monoclonal antibodies directed against $\beta(1-6)$ -branched $\beta(1-3)$ -glucans were characterized by ELISA with respect to crossreactions with $\beta(1-3)$ -, $\beta(1-6)$ -, $\beta(1-4)(1-3)$ -glucans, *Candida albicans* and *Cryptococcus neoformans* cell wall fragments. The presence of a β glucan epitope on the surface of the cell wall of *Candida albicans*, *Candida*
 15 *parapsilosis*, *Candida glabrata*, *Candida krusei*, an uncapsulated mutant of *Cryptococcus neoformans* was investigated by immunofluorescence microscopy.

We present what to our knowledge is the first mAb (A10A) that reacts with a $\beta(1-3)(1-6)$ -glucan epitope on the intact cell surface of *Candida*.

By a $\beta(1-3)$ -glucan associated epitope is meant an epitope which is present in
 20 $\beta(1-3)$ -glucans, and $\beta(1-3)(1-6)$ -glucans.

By a $\beta(1-3)(1-6)$ -glucan associated epitope is meant an epitope which is present in $\beta(1-3)$ -glucans, $\beta(1-6)$ -glucans, and $\beta(1-3)(1-6)$ -glucans.

Materials and Methods

25 Strains and condition of growth

Candida albicans ATCC 64549, *Candida glabrata* ATCC 90030, *Candida parapsilosis* CCUG 37233, *Candida krusei* ATCC 6258, and an unencapsulated *Cryptococcus neoformans* strain 602 were cultivated in Sabouraud dextrose broth, at 37°C overnight. The conversion of yeast to germ tube and hyphal forms of *Candida*
 30 *albicans* was carried out by transferring the *Candida albicans* yeast cells to RPMI 1640 and cultivation at 37°C for 18h.

Antigens

Cell wall fragments

Cell wall fragments of *Candida albicans* (CaCW) and *Cryptococcus neoformans* strain 602 (CnCW) were prepared by treatment of the yeast cells by glass beads as described earlier (12). The glucan structure in CaCW is composed of branching $\beta(1-3)(1-6)$ linkages. The cell wall of uncapsulated *Cryptococcus neoformans* is composed mainly of $\alpha(1-3)(1-4)D$ and $\beta(1-3)(1-6)$ -glucans (6).

Glucans

Glucan from *Saccharomyces cerevisiae* with $\beta(1-6)$ -branched $\beta(1-3)$ -linked glucose residues [$\beta(1-3)(1-6)glu$], *Alcaligenes faecalis* curdlan with (1-3)- β -linkages [$\beta(1-3)$], and glucan from barley with (1-4)(1-3)- β -linkages [$\beta(1-4)(1-3)$] were purchased from Sigma (St Louis, USA). Pustulan from lichen *Umbilicaria papulosa* with (1-6)- β -linked glucose residues [$\beta(1-6)$] was purchased from Calbiochem (San Diego, USA). According to the manufacturer pustulan contained only glucose. The purity of the glucans of baker yeast, curdlan, and barley were 98, 99 and 96% respectively, according to the specifications. Table 1 summarizes the trivial names, physical properties, and sources of the β -glucans used in this study. $\beta(1-3)(1-6)glu$, $\beta(1-4)(1-3)$, and $\beta(1-3)$ were dissolved in 0.3M NaOH at a concentration of 20 mg/ml. $\beta(1-6)$ was dissolved in water at 100°C at a concentration of 20 mg/ml.

Table 1

Structural and physical properties of β -glucans used in this study

Trivial name	Type of linkages	Source	Molecular weight	Solubility in water	Linear/ branched
Yeast glucan	$\beta(1-3)(1-6)$ -D-	<i>Saccharomyces cerevisiae</i>	17,000	insoluble	branched
Curdlan	$\beta(1-3)$ -D-	<i>Alcaligenes faecalis</i>	294,000	insoluble	linear

Barley	$\beta(1-4)(1-3)$ -D-	Barley plant	23,000	insoluble	linear
Pustulan	$\beta(1-6)$ -D-	<i>Umbilicaria</i> <i>papulosa</i>	20,000	soluble	linear

Antibodies to β -glucan

Production of mAbs

For the production of mAbs female Balb/c mice (6-8 weeks old) were immunized intraperitoneally (i.p) with either 50 μ g of $\beta(1-3)$ (2 mice), $\beta(1-3)(1-6)$ glu (4 mice) or 2.5×10^7 cells of formaldehyde treated uncapsulated *Cryptococcus neoformans* (4 mice) suspended in 200 μ l PBS containing 1 μ g of cholera toxin, which was used as an adjuvant (23). Two and four weeks later, the mice received intraperitoneal injections with the same amount of antigen. One week after the last injection, blood was collected and the antibody response to $\beta(1-3)(1-6)$ glu analyzed by ELISA. After an additional week another injection with the same amount of antigen was given, and three to four days later the animals were killed and their spleens used for fusion.

Myeloma cells were cultured in Iscoves medium supplemented with 2mM L-glutamine, penicillin (100 U/ml), streptomycin (100 μ g/ μ l) and 1% (w/v) fetal bovine serum (growing medium). Cell fusion and selection of hybrids were carried out as described by Köhler and milstein (11). Spleen lymphocytes from immunized mice were fused with SP2/0 murine myeloma cells at a 5:1 ratio using PEG 1500 (Boehringer Mannheim GmbH, Mannheim, Germany) as the fusing agent. The fused cells were distributed in 96-well culture plates at an approximately density of 4×10^5 cells in 200 μ l HAT selection medium (growing medium supplemented with hypoxanthin, aminopterin, thymidine). On day 10 post-fusion, the culture supernatants were screened for the presence of antibodies specific to $\beta(1-3)(1-6)$ glu and $\beta(1-3)$ by ELISA. Positive hybridomas, which all were of IgM isotype as determined by ELISA, were cloned by limiting dilution on a feeder layer of Balb/c peritoneal macrophages. Cells were grown in HAT medium for two weeks. The HAT was substituted by HT medium (growing medium supplemented with hypoxanthin and thymidine). Positive clones were cultivated in serum free medium HYQ-CCM1TM (from Hyclone Laboratories Inc, Utah, USA).

MABs were purified by $(\text{NH}_4)_2\text{SO}_4$ precipitation followed by affinity chromatography on agarose gel with covalently linked IgG goat anti-mouse IgM (Sigma, St

ELISA

Microplate wells (Nunc immunoplate, Denmark) were coated with 100 μ l of a 50 μ g/ml solution of β (1-3), β (1-4)(1-3), β (1-6), CaCW or CnCW and a 20 μ g/ml of β (1-3)(1-6)glu solution containing 50 mM Na₂CO₃ buffer, pH 9.3. The plates were incubated at room temperature (r.t.) for two hours and thereafter kept at 4°C overnight. After rinsing the plate once with PBS, 100 μ l of blocking buffer (BF) (1% BSA in PBS) were added to each well and the plate incubated for 1h at r.t. The plate was rinsed once with PBS. mAbs diluted in 1/10, 1/50, 1/100 and 1/1000 in PBS, were added to each well (100 μ l) and incubated for two hr at r.t. Hereafter the plate was rinsed three times with 0.05% TWEEN-20™, a polyoxyethylene sorbitol ester with a calculated molecular weigh of 1,225 daltons (also known as polyoxyethylenesorbitan monolaurate, Sigma, St. Louis, MO) in PBS (PBS-T) between each incubation step. Biotinylated rabbit anti-mouse IgM (DAKO, Glostrup, Denmark) diluted 1/5000 in PBS-T was added to the wells (100 μ l). The plate with monoclonal antibodies was further incubated at r.t. for 2h, and thereafter 100 μ l of alkaline phosphatase conjugated EXTRAVIDIN™, a form of avidin that combines high specificity and affinity of avidin for biotin with low non-specific binding at physiological pH (Sigma, St Louis, USA) diluted 1/1000 in PBS-T were added and the plate was incubated at r.t. for 60 min. Para-nitrophenylphosphate (1mg/ml, Sigma, St Louis, USA) diluted in diethanolamine buffer (pH 9.8) was added to each well and the absorbance was read at 405 nm when a suitable color had developed.

Inhibition-ELISA

Increasing amounts of $\beta(1-3)(1-6)\text{glu}$, $\beta(1-3)$, $\beta(1-4)(1-3)$, $\beta(1-6)$, CaCW, CnCW (1-1000 $\mu\text{g/ml}$) were added to series of tubes containing a constant amount of mAb or rabbit serum. The mAbs were also incubated with monosaccharide; β -D-glucose, glucose amine and mannose or disaccharides; trehalose with $\alpha(1-1)$, maltose

10

15

Example 1

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[illegible]

ity of A10A and B3B against $\beta(1-3)(1-6)\text{glu}$ and $\beta(1-3)$, $\beta(1-4)(1-3)$, $\beta(1-6)$, CnCW, and CaCW were studied (Fig. 1). A10A showed a high antibody activity against all antigens except for $\beta(1-6)$, and $\beta(1-4)(1-3)$. B3B showed an overall lower activity against the antigens. The highest antibody activity was obtained against CaCW followed by CnCW. It was intermediate against $\beta(1-3)$ and low against $\beta(1-3)(1-6)\text{glu}$, while it was not active against $\beta(1-6)$ and $\beta(1-4)(1-3)$. The highest antibody activity for both mAbs was found against CaCW. In addition, A10A showed a high activity against $\beta(1-3)(1-6)\text{glu}$.

The cross-reaction between $\beta(1-3)(1-6)\text{glu}$ or CaCW and the various glucan
10 antigens were studied by inhibition-ELISA.

It was found that the EI₅₀ of A10A for the homologous antigen, β(1-3)(1-6)glu, and CnCw were almost identical (6 and 5 µg/ml, respectively) (Table 1). EI₅₀ for β(1-3) and β(1-6) was 7- fold higher. EI₅₀ for β(1-4)(1-3) and CaCw was more than 60 and 40 times higher respectively, than the β(1-3)(1-6)glu or CnCw.

Table 1

Inhibition of the anti- $\beta(1-3)(1-6)$ glu and CaCW antibody activities of A10A by absorption with $\beta(1-3)(1-6)$ glu, $\beta(1-3)$, $\beta(1-4)(1-3)$, $\beta(1-6)$, CaCW, and CnCW. A10A was diluted 1/50

Absorbing agent	EI ₅₀ (μ g/ml) \pm Standard deviation	
	$\beta(1-3)(1-6)$ glu [#]	CaCW
$\beta(1-3)(1-6)$ glu	6 \pm 2	31 \pm 12
$\beta(1-3)$	40 \pm 12	185 \pm 170
$\beta(1-4)(1-3)$	359 \pm 39	>*
$\beta(1-6)$	43 \pm 17	>*
CaCW	238 \pm 112	56 \pm 6
CnCW	5 \pm 2	6 \pm 4

#The absorbance value of the unabsorbed antibody was 1.8 against $\beta(1-3)(1-6)$ glu and 1.2 against CaCW.

* No inhibition at the highest concentration tested, 1000 μ g/ml.

The A10A activity against CaCW showed that CnCW was a 9-fold stronger inhibitor than the homologous antigen (Table 1). In addition, $\beta(1-3)(1-6)$ glu was also stronger as inhibitor than CaCW. EI₅₀ for CaCW was almost twofold higher (56 μ g/ml) than that of $\beta(1-3)(1-6)$ glu (31 μ g/ml). Thus, the A10A epitope involved the branching region of the glucan, the $\beta(1-3)(1-6)$ linkage, which was available to a higher extent in CnCW than in CaCW.

The specificity of B3B to CaCW was analyzed by inhibition-ELISA. The EI₅₀ for CaCW and $\beta(1-3)$ was roughly the same and they were more than 15 times higher than that of EI₅₀ for $\beta(1-3)(1-6)$ glu (Table 2). $\beta(1-4)(1-3)$ and CnCW did not inhibit the anti-CaCW antibody activity at the highest concentration tested. The EI₅₀ for $\beta(1-6)$ was almost 40-fold higher than that of $\beta(1-3)(1-6)$ glu. This inhibition pattern of B3B differed from that of A10A by the lack of inhibitory effect of CnCW, while still being inhibited by $\beta(1-3)(1-6)$ glu. Thus, the B3B epitope was highly exposed by the free form of $\beta(1-3)(1-6)$. None of the mono- and disaccharides inhibited the anti- $\beta(1-3)(1-6)$ glu antibody activity of the two mAbs.

Table 2

Inhibition of the B3B anti- CaCW antibody activity by absorption with $\beta(1-3)(1-6)$ glu, $\beta(1-3)$, $\beta(1-4)(1-3)$, $\beta(1-6)$, CaCW, and CnCW. The mAb was diluted 1/20 in in PBS containing 0.1% BSA. The absorbance value was 0.4 of the unabsorbed antibody.

Absorbing agent	El ₅₀ (μg/ml)
β(1-3)(1-6) glu	20
β(1-3)	450
β(1-4)(1-3)	>*
β(1-6)	750
CaCW	306
CnCW	>*

*> , no inhibition at the highest concentration tested (1000μg/ml).

Example 2

Availability of β(1-3)(1-6) epitopes on the cell surface of *Candida* and *Cryptococcus neoformans*

The availability of β(1-3)(1-6)-glucan for antibody binding on the cell surface of various *Candida* species and *Cryptococcus neoformans* were analyzed by IF microscopy using A10A and B3B. Yeast and mycelial forms of *Candida albicans*, *Candida parapsilosis*, *Candida krusei*, *Candida glabrata* and the uncapsulated mutant of *Cryptococcus neoformans* were all positive by IF (Fig. 3). The intensity of fluorescence differed depending on the morphology and distribution of the antigens in the cell wall. Uncapsulated *Cryptococcus neoformans* was strongly immunoreactive with A10A. This mAb also stained the yeast and mycelial forms of *Candida albicans*, but somewhat weaker. The other species of *Candida*, *Candida parapsilosis*, *Candida krusei* and *Candida glabrata* were all stained with A10A.

B3B did not stain any of the fungal strains.

Discussion

While A10A reacted with an epitope exposed on the cell surface of intact fungal cells, the other one recognized an epitope present in the cell wall fragments only. Both mabs reacted with the free form β(1-3) or β(1-3)(1-6)-glucan. The mycelial form of *Candida albicans* was stained with A10A to the same degree as the yeast form as shown by IF.

The novel A10A mAb also recognized an epitope present in β(1-6). MAbs directed against β(1-6) and β(1-3)(1-6)-glucans have been described earlier (4, 22). The mAb directed against β(1-6) as obtained by immunization with Zymolyase extract

from *Candida albicans* was shown not to react with epitopes on the cell wall of *Candida albicans* unless the outer layer, being formed by mannoproteins, was disrupted by the effect of tunicamycin. Tunicamycin interferes with the N-glycosylation of proteins so that new synthesis of mannoproteins will not become glycosylated during cell growth (10). Thus, A10A as well as B3B differed from this $\beta(1-6)$ epitope binding monoclonal. Regarding the other two reported mAbs, one was suggested to be mainly directed against $\beta(1-3)$ in Schizophyllan ($\beta(1-3)$ -glucan with branching $\beta(1-6)$ glucose residues) (5), while the detailed specificity of the other one with regard to various glucans was not reported. The presence of those epitopes on the cell surface of fungi was, however, not studied. The mAbs were produced for measuring either schizophyllan in serum during treatment by this agent as an anti-cancer drug, or for determining the immunological properties of another anti-tumor polysaccharide containing $\beta(1-3)$ and $\beta(1-6)$ -glucans (5). Thus, in two of the first described monoclonals none was analysed for binding activities against native $\beta(1-3)$ glucans exposed in the cell wall fragments of *Candida* or *Cryptococcus* as was shown for A10A and B3B. In order to perform analyses on natural components such as intact cells, cell wall fragments, or shed $\beta(1-3)$ glucans, only our mabs are characterized with respect to this.

Our second mAb B3B did not recognize cell wall antigens in indirect IF and only weakly in inhibition-ELISA. Most probably the explanation for this is the presence of the epitope mainly in the deeper parts of the cell wall and thereby not available on the cell surface of the intact cell. Yet another explanation could be that it only recognizes a particular form of the glucan antigen, since the weak anti-CaCW activity of B3B was inhibited by $\beta(1-3)(1-6)$ -glucan at a low concentration. Although B3B was produced against $\beta(1-3)$, the EI_{50} for this glucan regarding B3B anti-CaCW activity was approximately 15 times higher than that of $\beta(1-3)(1-6)$. The fact that $\beta(1-3)$ is linear and $\beta(1-3)(1-6)$ is branched in addition to a 10 times higher molecular weight than $\beta(1-3)(1-6)$ may have influenced the epitope density. It is also known that the ultrastructure of higher molecular weight β glucans exhibits various forms such as single-helical, triple-helical, and a mixture of both, due to interchain hydrogen bonding between each main chain of polyglucose residues (25). Lower molecular weight β glucans adopt a randomly coiled form in aqueous solution (1). The percentage of branching, i.e. the number of (1-6)- per (1-3)-linkage may also differ between differ-

ent fungal species. The availability of epitopes may be higher in randomly coiled regions of branched β glucans.

During growth medically important fungi seem to shed $\beta(1-3)$ -glucan into the culture medium. The concentration of $\beta(1-3)$ -glucan in serum from patients with deep fungal infections can be very high as determined by the G factor based *Limulus* assay (16, 17, 21). We have found $\beta(1-3)$ -glucan in serum of all patients with candidemia, but in none of women with superficial *Candida* infection, or healthy controls (13). Thus, $\beta(1-3)$ -glucan seems to be a sensitive assay. However, since also other types of glucans may activate the *Limulus* assay (24b) an immunoassay based on two specific antibodies would be more specific. Two assays have been reported for the determination of $\beta(1-3)$ glucan levels: the first utilizes a monoclonal IgG antibody specific for consecutive alignments of $\beta(1-3)$ -D-glucopyranosyl residues and biotinylated horse-shoe crab protein, T-GBP, from *Tachypleus tridentatus* (24), while the second assay employs a high affinity receptor (galactosyl ceramide) for $\beta(1-3)$ glucans and a mAb that is described as being specific for complex fungal cell wall $\beta(1-3)$ glucans (15). The T-GBP-protein – based sandwich ELISA was shown to react readily with $\beta(1-3)$ glucans including barley $\beta(1-4)(1-3)$ (24). The other immunoassay, based on the capture agent galactosyl ceramide, was shown to not react with $\beta(1-3)$ glucan, a glucan which readily reacts with our mAbs. Furthermore in that report no analyses were performed with *Candida* or *Cryptococcus* whole cells or cell wall fragments.

The presence of $\beta(1-3)$ -glucans in serum of patients with deep fungal infections may be a useful marker for laboratory diagnosis of these infections. Future investigations will address the usefulness of our mAbs to glucan in an immunoassay-based kit for the rapid detection of $\beta(1-3)$ glucans in blood samples, in other specimens from patients with invasive fungal infections, or in other body fluids such as mucosal secretions and urine. Moreover, the presence of the $\beta(1-3)$ or $\beta(1-3)(1-6)$ glucan epitope on the intact surfaces of both *Candida* species and non-encapsulated *Cryptococcus neoformans*, as seen with A10A, or in cell wall fragments as seen with both A10A and B3B has not been reported earlier regarding activities of monoclonal antibodies directed against $\beta(1-3)$ glucans.

The antibodies according to the invention can be used for the detection of free, cellwall associated-, and/or cell surface-associated $\beta(1-3)$ glucans utilizing immuno-

assays or immunohistology for the laboratory diagnosis of fungal infections. Further, they may be used in immunotherapy.

The antibodies disclosed in the present application may also be used for the detection of airborne mould, or mould present in dust or water, or in any other component. Thus, the antibodies according to the invention may be used for the detection of all kinds of medically important fungi, for example in connection to allergy problems and in the detection of house mould.